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By G. N. STEWART, M.D., and T. F. ZUCKER, M.S.

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THE ACTION OF HYDROCELE FLUID AND CERTAIN OTHER PATHOLOGICAL LIQUIDS ON SOME OF THE OBJECTS USED IN BIOLOGICAL TESTS FOR EPINEPHRIN.*

BY G. N. STEWART, M.D., AND T. F. ZUCKER, M.S.

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PLATES 22 TO 27.

The question whether the tone-increasing action of serum on intestine and uterus segments is due altogether, or mainly, to the same property of the serum which causes it to constrict artery rings and to slow the stream through the frog perfusion preparation of Laewen[1] was tested by comparing the effect of hydrocele fluid on the four preparations. The typical hydrocele fluid is a transudate which, although containing fibrinogen, does not clot spontaneously, or at least not readily, but can be made to clot by the addition of thrombin or such liquids as blood serum which contain thrombin. Its poverty in thrombin is related to its poverty in formed elements. If the development of the property in shed blood by which it causes constriction of blood vessels is connected with changes in the formed elements, this property should be absent or very slightly marked in hydrocele fluid. If in spite of a negative result with blood vessels hydrocele fluid were found to cause an action on intestine and uterus segments comparable in magnitude to that of serum, the conclusion would be decidedly strengthened that the action of serum on these latter objects is different in nature from its action on the blood vessel preparation. In the following experiment these expectations were realized.

Hydrocele fluid was obtained with such precautions as insured the complete absence of blood. The fluid was the typical non-inflammatory hydrocele fluid, perfectly clear and yellowish in color, and it

* Received for publication, November 2, 1912.

[1] Stewart, G. N., and Zucker, T. F., *Jour. Exper. Med.*, 1913, xvii, 152.

remained free from the slightest clot for the whole period (over a week) during which it was kept in the ice chest. It was put on ice a very short time after being drawn. The total proteins were determined by precipitation with excess of alcohol.[2] The determination was made in duplicate on two samples of fifty cubic centimeters each. One yielded 2.37 grams, the other 2.38 grams; the protein content of 100 cubic centimeters was, therefore, 4.75 grams.

RESULTS.

Artery Rings.—Hydrocele fluid caused no noticeable effect when it replaced Ringer solution (figure 1, observation 8), while serum from a patient in diabetic coma replacing the hydrocele fluid (observation 9) caused a prompt and large constriction.

Frog Perfusion Preparation.—The slowing produced by the hydrocele fluid was slight and transient in comparison with that caused by dog serum, and the original outflow was quickly and completely restored (figure 2). The initial elevation on the curve is simply due to the mechanical increase of pressure caused by the injection.

Rabbit Intestine Preparation.—The increase of tone caused by the hydrocele fluid when it replaced Ringer solution was not far from equal to that caused by serum from the rabbit's own blood (figure 3, observations 2 and 6). This is shown even more strikingly in figure 4, where at 8 rabbit serum replaced Ringer solution and was itself replaced at 9 by hydrocele fluid. Instead of a fall, as would have been the case had the tone-increasing power of the hydrocele fluid been decidedly inferior to that of the serum, a sharp rise is seen at 9 due probably merely to the renewal of the liquid, and the curve then soon falls to the same height as before 9. When the observations were made in the reverse order, an almost identical curve was obtained. At 11, figure 4, Ringer solution was replaced by hydrocele fluid and the fluid at 12 by rabbit serum. The same transient rise occurred at 12 followed by a fall to the level which was being maintained before 12, indicating that the hydrocele fluid and serum were about equally potent in maintaining the increased tone.

That the actual change of fibrinogen into fibrin has little, if any-

[2] Hoppe-Seyler, F., Handbuch der physiologisch- und pathologisch-chemischen Analyse, 8th edition, Berlin, 1909, 662.

thing, to do with the tone-increasing property of shed blood for the intestine segments is illustrated by observations on a pleuritic exudate obtained from the right pleural cavity of J. S., a man aged twenty-two years. He had been ill for three weeks and complained of pain in the right side. The fluid was obtained free from blood and soon clotted into a jelly so that it could not be poured out of the bottle. The liquid from this clot was compared in a series of observations on the rabbit intestine segment with blood serum from the same rabbit. In figure 5 at 8 Ringer was replaced by the pleural liquid, and at 10 Ringer was replaced by rabbit serum. The increase of tone in 10 was distinctly greater than in 8. This result was obtained repeatedly on the same segment. Thus hydrocele fluid, in which no fibrin had been formed, had a tone-increasing power on the intestine equal to that exerted by rabbit serum; while the pleural liquid, after the formation of much fibrin, was inferior to rabbit serum in this regard. The liquid was richer in protein than the hydrocele fluid, containing 3.081 and 3.069 grams in two portions of fifty cubic centimeters, *i. e.*, 6.15 grams in 100 cubic centimeters. The pleuritic exudate probably was, or had been, richer in formed elements than the hydrocele fluid, so that if cell decomposition or changes of permeability in the cells are responsible for the tone-increasing property as regards the intestine that property ought to have been more strongly developed in the pleuritic fluid.

That the change of fibrinogen into fibrin is not necessarily associated with the development of the pressor property of shed blood for artery rings, any more than with its tone-increasing power for intestine and uterus, was demonstrated by observations on fluid from a cyst of the suprarenal gland. This fluid was unclotted when obtained, but could be made to clot in the thermostat. The action of the liquid before and after clotting was compared on sheep artery rings and rabbit intestine and uterus segments (figures 6 to 9), and was found to be practically identical. This was not because the epinephrin which it contained caused an effect so great as to obscure any difference which might have existed. For it was shown that the concentration of epinephrin present, although considerable (probably somewhat less than 1:1,000,000), produced a much

smaller pressor effect on the rings and a much smaller increase of tone in the uterus segment than did the cyst liquid. The extremely prompt rise in the case of the uterus preparation, which is by no means peculiar to the action of this liquid, but is characteristic of the reaction of the uterus segments to serum, precludes the possibility that the tone-increasing property of the liquid is developed after contact with the tissue. It is only a matter of seconds when the rise begins. Further, after emptying the apparatus no clot could be seen.

Incidentally we have here an example of a plasma (an unclotted serous liquid) which presented no advantages over the corresponding serum (the cyst liquid after coagulation) for epinephrin-testing.

Rabbit Uterus Preparation.—On the uterus preparation of the same rabbit, a young non-pregnant adult whose intestine had been used for testing the hydrocele fluid, the tone-increasing power of the hydrocele fluid was distinctly less than that of the rabbit serum, so that when the hydrocele fluid was caused to replace the serum at 19, figure 10, the tone of the preparation was decidedly diminished. When hydrocele fluid replaced Ringer solution the increase of tone was inferior to that occasioned by the rabbit serum. Adrenalin 1:5,000,000 caused an increase of tone after a slight preliminary inhibition, adrenalin 1:50,000 a marked increase of tone without preliminary inhibition. After being kept four days in the ice chest, during which time it remained perfectly clear and there was no sign of bacterial growth, the hydrocele fluid was again tested on the uterus segment from an adult non-pregnant rabbit (figure 11). When the hydrocele fluid replaced Ringer, a rise of tone (observation 38) was obtained of about the same magnitude as that caused by adrenalin 1:1,000,000 (observation 41) or of hirudin whole blood from a dog (observation 30). The rise occasioned by the hydrocele fluid in this observation appears to be much more transient than is really the case, since the drum was moving more slowly than in the other observations. For the sake of comparison, observation 28, showing the effect of hirudin defibrinated blood replacing Ringer solution, is also reproduced. Observation 32 shows the action of the undiluted dog serum, without hirudin, and observation 34 that of the serum diluted with its own

volume of Ringer. The hydrocele fluid produces a maximum increase of tone fully equal to that given by the diluted serum, although not quite so durable. It will be remarked that as regards its protein content it also resembles a somewhat diluted serum. This agrees very well with the view that the action of the hydrocele fluid on the intestine and uterus is mainly a general nutritive action of the same nature as that of the serum. If we conceive, on the other hand, the serum effect as being due to a special substance originally present in the blood plasma, it would be necessary to assume that this substance in passing into the hydrocele fluid was diluted in approximately the same degree as the plasma proteins.

With rings of sheep artery dog serum, without hirudin, and hirudin serum gave a very good rise. The dog hirudin plasma also caused a good rise, although not so great as that given by the sera.

The results of all the observations on the hydrocele fluid strengthened the conclusion that the constrictor action of serum for blood vessels is not a general action of the serum common to other serous liquids, but that it is due to a property developed in the shed blood which has a special relation to blood vessels.

Some observations were also made on ascitic fluid obtained twice, at an interval of seven weeks, from the same patient.

J. M., a man 50 years old, was admitted on July 6, 1912, to the City Hospital, suffering from cardiorenal disease (mainly renal) with a high systolic pressure (200 mm. of mercury), and general edema involving the face and the upper and lower limbs. The urine constantly contained albumen and casts. The left border of the cardiac dullness was two fingers' breadth outside the nipple line. On auscultation at the apex the first sound was impure, the second sound loud. In the aortic area the second sound was much accentuated and the pulmonary second was also accentuated. The abdomen was distended with free fluid.

On July 11, eight liters of liquid were removed from the abdomen, on August 19 more than seven liters, and on August 28, nearly two liters. The specimens were obtained quite free from blood. The action of the liquid obtained on July 11 and August 28 was determined on artery rings, and on segments of intestine and uterus. The protein in the fluid of July 11 was 0.637 gm. and 0.623 gm. in two portions of 50 c.c., *i. e.*, 1.26 gm. in 100 c.c. In the specimen of August 28 somewhat more protein was present, 1.055 and 1.029 gm. in two portions of 50 c.c., *i. e.*, 2.084 gm. in 100 c.c. Neither specimen showed any trace of clotting, although kept for several days.

With the artery rings no rise whatever was caused (figure 1, observation 10) whereas a serum used for comparison produced a very good rise (observation 11).

On the intestine preparation the effect was an increase of tone accompanied by an increase in the size of the rhythmical contractions. The effect was qualitatively similar to that produced by serum from the same patient, as shown in figure 12, for which the material of July 11 was used. At 4 Ringer solution was replaced by the ascitic fluid, and at 6 by serum from the patient's clotted blood obtained at the same time. The tone-increasing effect of the undiluted serum was decidedly greater than that of the peritoneal liquid.

Observations 8 to 14 (figure 12) were made with the object of determining in what dilution the serum effect would be approximately equal to that of the liquid. At 8 Ringer solution was replaced by serum diluted with two volumes of Ringer, at 12 by serum diluted with five volumes of Ringer, at 14 by ascitic fluid. The effect produced by the fluid was not very different, as regards increase of tone, from that produced by the serum diluted with five volumes of Ringer, and was superior, as regards increase in the size of the beats. Although no great stress should be laid on the coincidence, it may be noted that here also the pathological serous liquid exerted an action which, as compared with that of the serum, was approximately proportional to its content of protein.

On the uterus preparation (figure 13) from an adult non-pregnant rabbit the ascitic fluid (of July 11) produced a good increase of tone followed by complete relaxation (observation 21). The relaxation was especially evident when the fluid was added at a time when the uterus segment possessed a high initial tone (observation 38). At 39 it was particularly observed that relaxation was complete. The relaxation following the increase of tone was equally complete in observation 21 where the initial tone was low. The contractions ceased entirely during the relaxation.

On the uterus preparation from another rabbit (non-pregnant, small, and immature) the same phenomenon of relaxation with cessation of contraction was observed (figure 14, observation 24) succeeding a preliminary increase of tone. On the same segment the serum of the patient caused a good rise of tone which was sustained (observation 22).

The specimen of ascitic fluid obtained from the same patient on

August 28 caused a fair increase of tone in a rabbit intestine segment, although distinctly less than that produced by dog serum diluted with five volumes of Ringer solution.

On the uterus preparation from an adult non-pregnant rabbit the same inhibition succeeding the increase of tone which was observed with the other specimen of the fluid from this patient was noted. We are not in a position to state what the cause of this inhibition was. The other transudate examined (hydrocele fluid) also exhibited this effect, although to a smaller extent. It is a phenomenon which we have not observed with blood serum or plasma and is probably due to some constituent of the pathological liquids not present, or present in much smaller amount in blood. However this may be, the uterus observations again illustrate the point on which we have more than once insisted elsewhere, that we cannot postulate without experiment a similar action of a given serous liquid on different test objects any more than we can postulate a similar action of different serous liquids on one and the same test object. Each liquid must be investigated separately for each test object.

This point is well illustrated by the tracings reproduced in figures 15 and 16 in which the action of blood serum from a fatal case of diabetic coma is compared with that of dog serum (of September 12). In figure 15 with a rabbit intestine preparation, Ringer solution was replaced at 14 by the dog serum and at 16 by the diabetic serum. In figure 16 with the uterus preparation from the same rabbit (a non-pregnant adult), Ringer solution was replaced at 36 by the diabetic serum and at 38 by the same dog serum as was used in figure 15. The increase of tone in the uterus segment caused by the diabetic serum was much greater than that produced by the dog serum, whereas the dog serum occasioned a greater increase of tone in the intestine segment than the diabetic serum. On the artery rings the diabetic serum caused an unusually prompt and large effect.

The diabetic serum was obtained from a young woman twenty years old, a telephone operator. She collapsed from overwork and was treated by her physician for nervous breakdown for some weeks before she applied for treatment at Lakeside and Western Reserve University dispensary. On admission at the dispensary she showed tachycardia, exophthalmos, slight thyroid enlargement, and loss of weight. Sugar was present in the urine to the extent of 3 per cent.

The diagnosis made was Graves' Disease with glycosuria. She was put on a non-carbohydrate diet, and returned the following week slightly improved. There was still some sugar in the urine. The same treatment was continued. She returned the next week and said she was worse and entered the hospital, where she quickly developed well marked coma with a distinct odor of acetone in the breath. The non-carbohydrate diet was cancelled, but the coma progressively deepened and she died about twelve hours later without regaining consciousness. Ten hours before death 100 c.c. of blood were removed preparatory to the giving of sodium bicarbonate intravenously. This was the specimen of blood tested.

We are indebted to the staffs of Lakeside Hospital and the City Hospital, especially to Dr. Bucher of the City Hospital, for the material investigated.

EXPLANATION OF PLATES.

All tracings are to be read from left to right. Time is marked in half minutes. Unless otherwise mentioned the liquid to be tested was always caused to displace the Ringer solution in which the preparation was immersed. Two c.c. of the liquid to be tested were always added, which was sufficient to displace the Ringer solution. The weight of the preparations, after drying with blotting paper, is given to facilitate comparison of the mass of tissue acted on, with the amount of active substance.

PLATE 22.

FIG. 1. Sheep artery rings. At 8 hydrocele fluid replaced Ringer, at 9 serum from a patient in diabetic coma replaced the hydrocele fluid. The slight rise in the curve after 8 does not indicate a genuine constriction due to the hydrocele fluid. It was probably caused by accidental jarring. At 10 ascitic fluid from J. M. replaced Ringer, and was itself replaced at 11 by dog serum. The rise produced by the dog serum is more abrupt than was observed with any other serum except that from the case of diabetic coma.

FIG. 2. Frog perfusion preparation. Curves showing changes in outflow with hydrocele fluid and dog serum, each diluted with three volumes of Ringer solution. The number of drops per half minute is laid off along the vertical, and the time in half minutes along the horizontal axis. Each small division corresponds to a half minute. Twenty-one drops correspond to 1 c.c.

FIG. 3. Rabbit intestine. Weight of preparation, 0.27 gm. At 2 hydrocele fluid, at 4 serum from the rabbit's blood, and at 6 hydrocele fluid replaced Ringer.

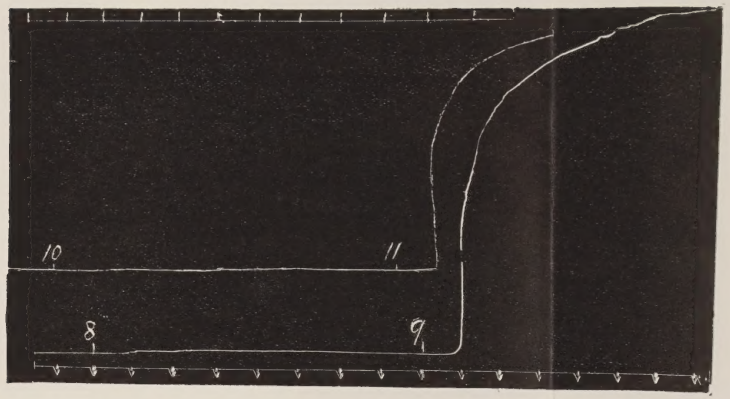


FIG. 1.

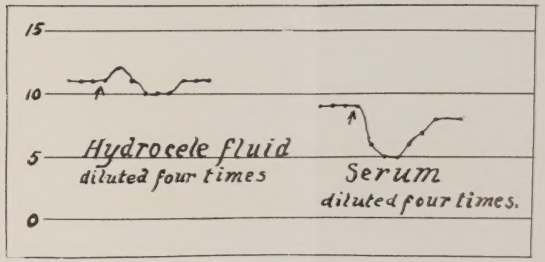


FIG. 2.

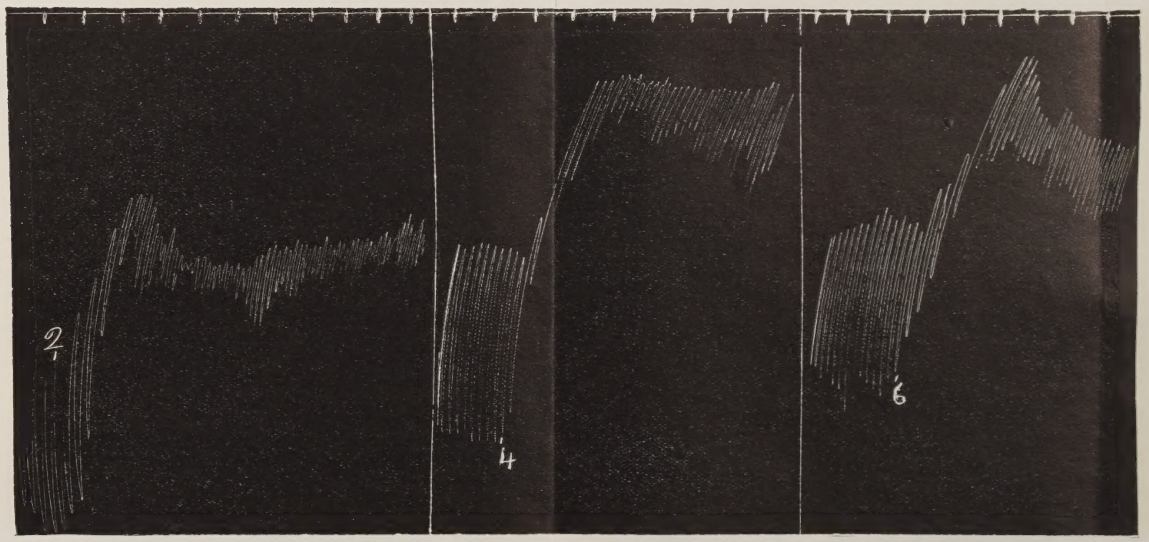


FIG. 3.

(Stewart and Zucker: Action of Hydrocele Fluid.)

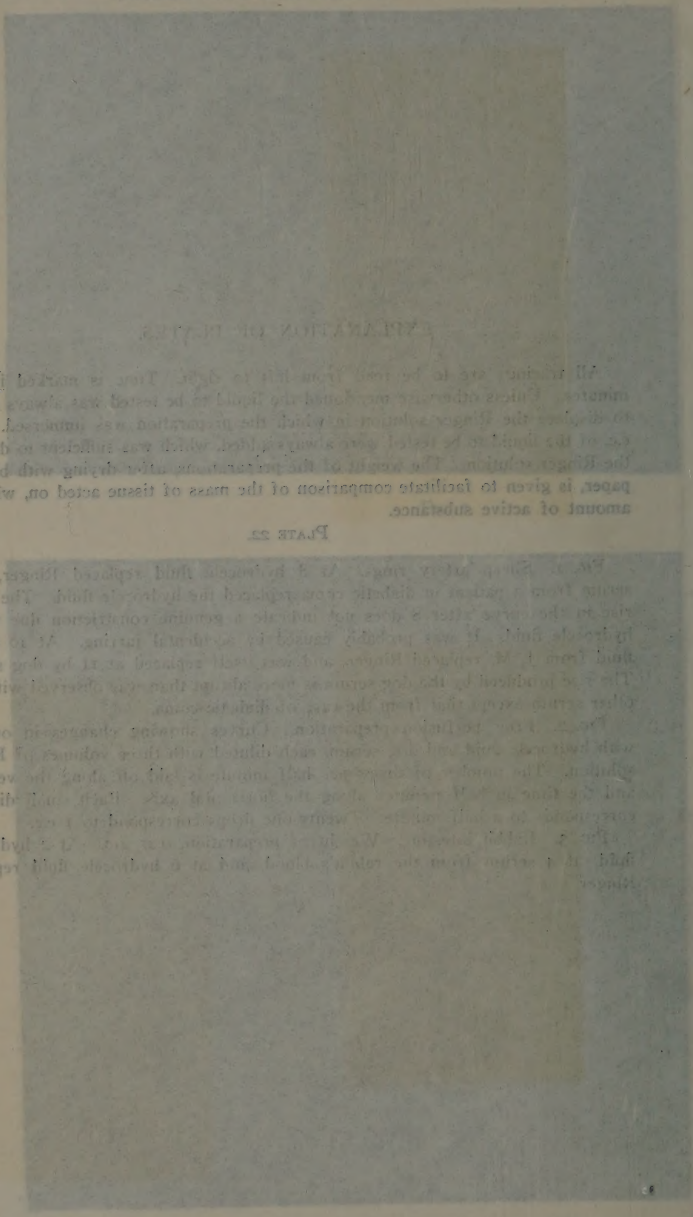


PLATE 23.

FIG. 4. The same intestine preparation as that used for figure 3. At 8 Ringer was replaced by the rabbit's own serum, and this at 9 by hydrocele fluid. At 11 Ringer was replaced by hydrocele fluid, and this at 12 by rabbit serum.

FIG. 5. Intestine. Weight of preparation, 0.34 gm. At 8 serum from clotted pleural fluid (from J. S.) replaced Ringer. At 10 rabbit serum replaced Ringer.

FIG. 6. Sheep artery rings. Fluid from cyst of suprarenal gland. At 3 Ringer was replaced by the fluid which had clotted at 4 by the unclotted fluid at 5 by adrenalin solution in Ringer, 1 : 50,000 (freshly opened).

FIG. 7. Intestine preparation. Weight of preparation, 0.30 gm. At 8 Ringer was replaced by unclotted adrenal cyst liquid, at 10 by cyst liquid after it had clotted.

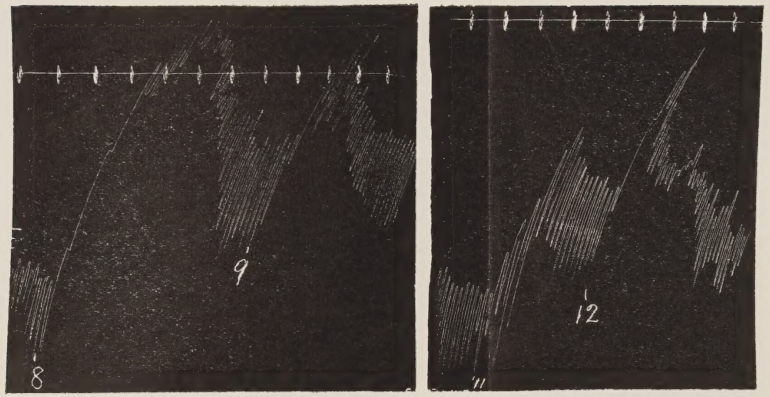


FIG. 4.

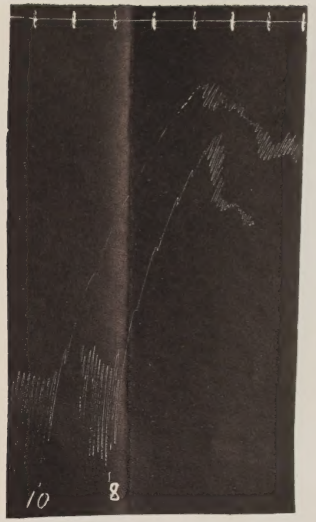


FIG. 5.

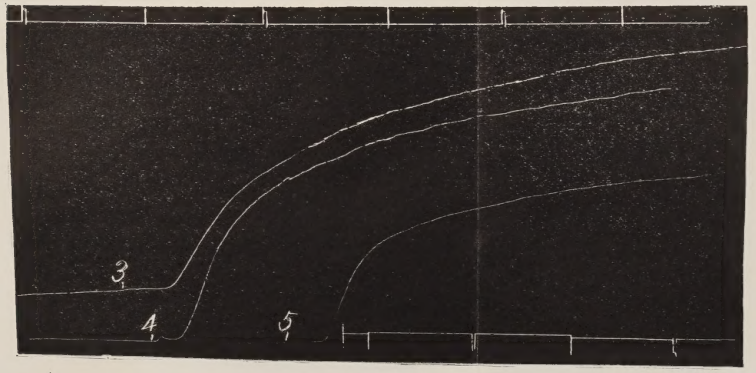


FIG. 6.

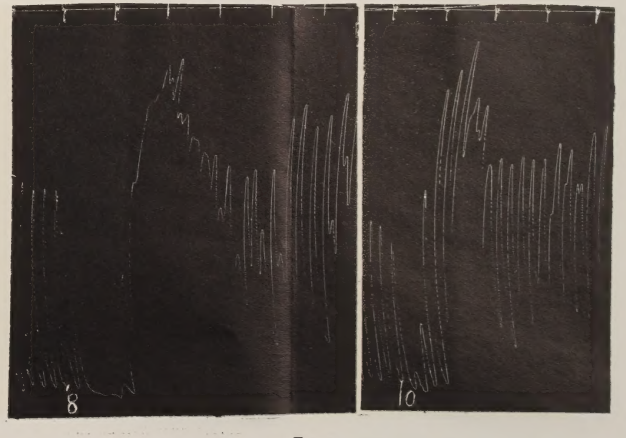


FIG. 7.

(Stewart and Zucker: Action of Hydrocele Fluid.)

PLATE 24.

FIG. 8. Same intestine preparation as used in figure 7. At 12 Ringer was replaced by the unclotted cyst fluid, at 14 by the cyst fluid after clotting. At 16 sheep serum replaced Ringer and was itself replaced at 17 by the unclotted cyst fluid.

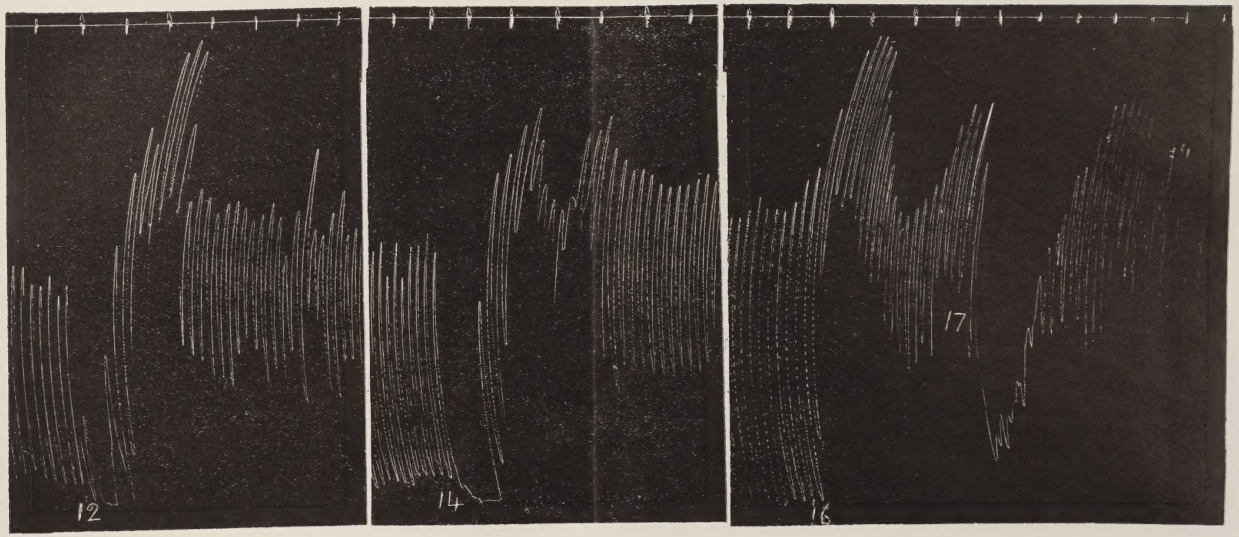


FIG. 8.

(Stewart and Zucker: Action of Hydrocele Fluid.)

PLATE 25.

FIG. 9. Uterus preparation from an adult non-pregnant rabbit. Weight of preparation, 0.30 gm. At 28 Ringer was replaced by the unclotted cyst liquid, at 30 by the cyst liquid after it had clotted. The writing point went a little above the drum and then gradually came down. At 32 Ringer was again replaced by the unclotted cyst liquid.

FIG. 10. Uterus from a young non-pregnant rabbit. Weight of preparation, 0.17 gm. At 18 Ringer was replaced by rabbit serum and this at 19 by hydrocele fluid. At 20 additional hydrocele fluid was run in. The magnification was two thirds of that usually employed.

FIG. 11. Uterus from an adult non-pregnant rabbit. Weight of preparation, 0.71 gm. Magnification 2½ times less than that usually employed. Comparison of hydrocele fluid with material obtained from a dog on Sept. 10. At 28 Ringer was replaced by the dog's hirudin defibrinated blood, at 30 by the dog's whole hirudin blood, at 32 by undiluted serum from the dog's clot, at 34 by serum from the dog's clot diluted with its own volume of Ringer, at 38 by hydrocele fluid, and at 41 by adrenalin 1 : 1,000,000 in Ringer solution. The time trace above observation 28 shows that the drum was then moving much more slowly than during the other observations.

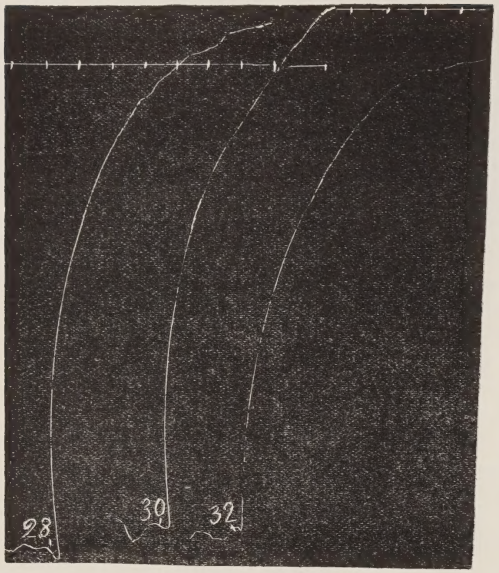


FIG. 9.

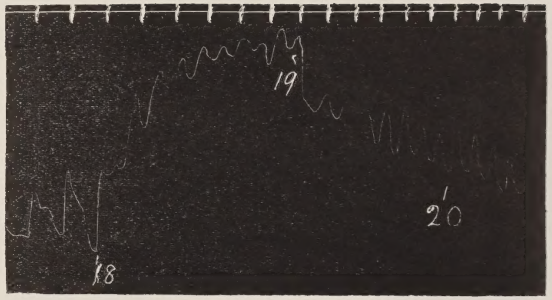


FIG. 10.

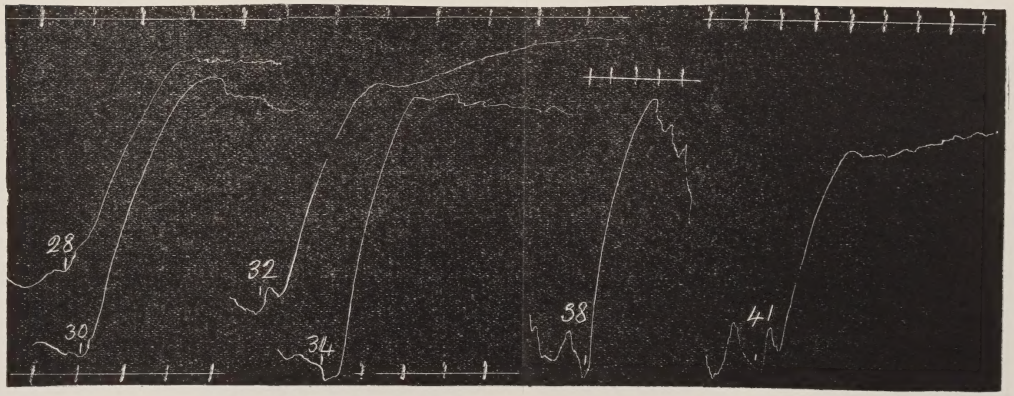


FIG. 11.

(Stewart and Zucker: Action of Hydrocele Fluid.)

PLATE 26.

FIG. 12. Intestine. Weight of preparation, 0.25 gm. At 4 ascitic fluid from J. M., at 6 serum from J. M., at 8 his serum diluted with two volumes of Ringer, at 12 his serum diluted with five volumes of Ringer, and at 14 his ascitic fluid replaced Ringer.

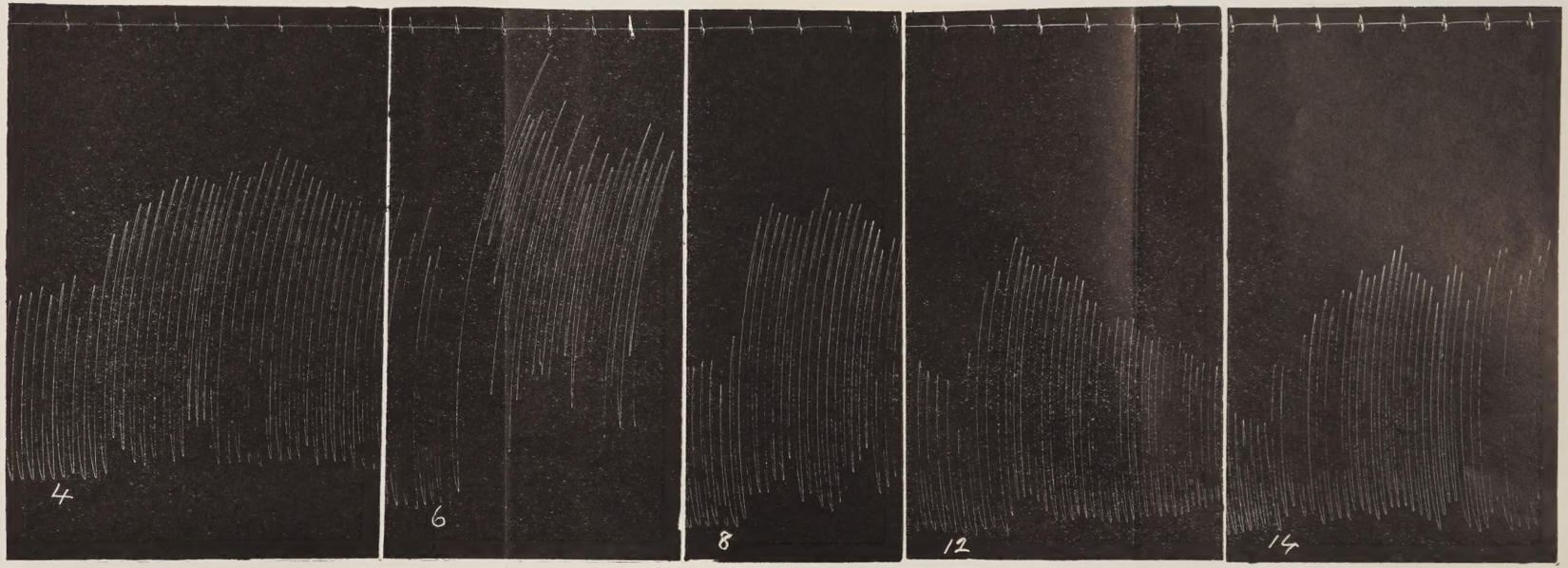


FIG. 12.

(Stewart and Zucker: Action of Hydrocele Fluid.)

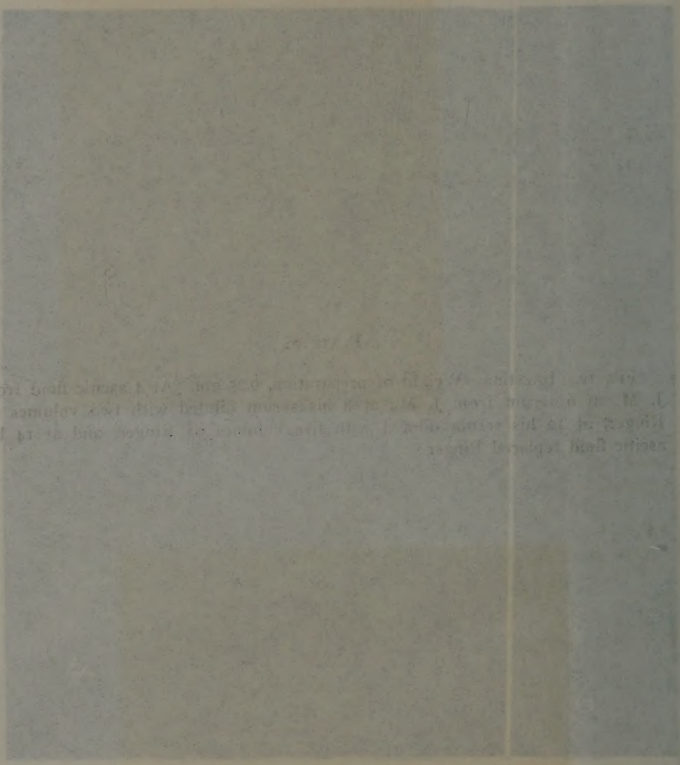


PLATE 27.

FIG. 13. Uterus from an adult non-pregnant rabbit. Weight of preparation, 0.55 gm. At 21 ascitic fluid from J. M. replaced Ringer. The initial tone of the uterus segment was small. At 38 the ascitic fluid replaced Ringer when the initial tone of the segment was high. At 39 the preparation was seen to be completely relaxed. The magnification was 2½ times less than that usually employed.

FIG. 14. Uterus preparation from a young non-pregnant rabbit. Weight of preparation, 0.45 gm. At 22 J. M.'s serum, diluted with five volumes of Ringer solution, and at 24 his ascitic liquid replaced Ringer.

FIG. 15. Intestine. Weight of preparation, 0.40 gm. At 14 serum from dog's clot, at 16 serum from a patient in diabetic coma replaced Ringer. The magnification was 2½ times less than that generally used.

FIG. 16. Uterus. Weight of preparation, 0.76 gm. At 36 serum from the patient in diabetic coma, and at 38 serum of the clot of the same dog that was used in figure 15 replaced Ringer. The magnification was the same as in figure 11.

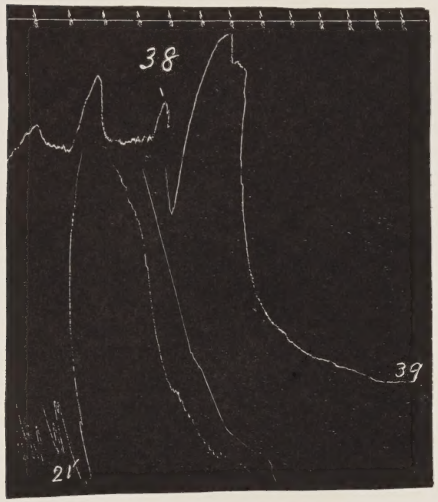


FIG. 13.

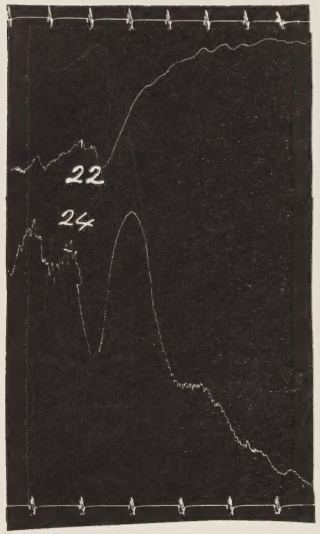


FIG. 14.

FIG. 15.

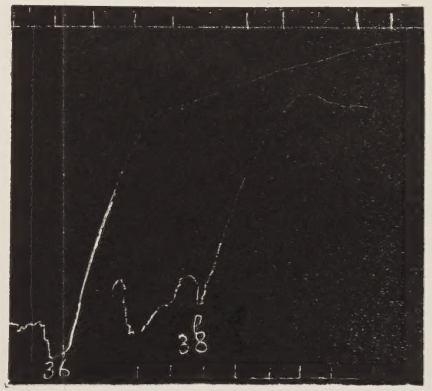
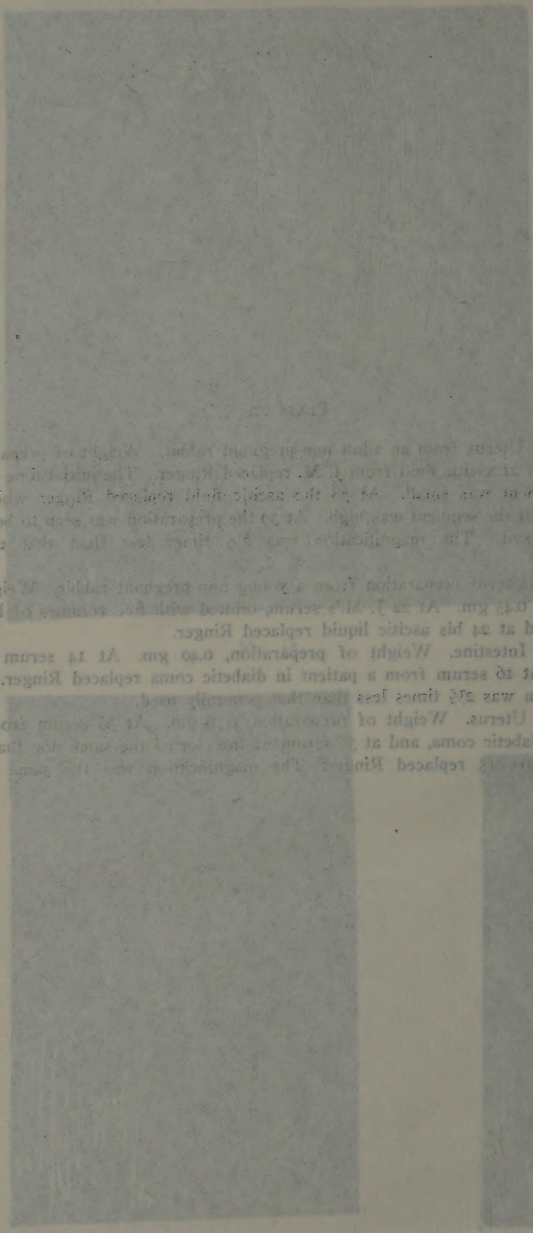


FIG. 16.

(Stewart and Zucker: Action of Hydrocele Fluid.)



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